

*Please replace the following paragraph starting on page 18, line 18 and ending on page 19, line 3
(TWICE AMENDED)*

Fig. 2 (SEQ ID NOS:2 and 3) shows a schematic diagram illustrating the test constructs generated, in which stable stem-loop structures were inserted into the Nco I site of the WT1 gene. A Sau 3AI fragment of the WT1 gene was inserted into pSP65(T), positioning a tract of 38 adenosine residues downstream of the WT1 gene, allows first strand synthesis to be primed by an oligo d(T) primer. Clones containing either one or two copies of the (M1/X) stem-loop structures were isolated and characterized by sequencing. Insertion of one or two copies of GNRA stem-loop structure was done in flWT1, a derivative of pSP/WT1 in which a portion of the GC-rich 5' UTR of WT1 was present. Termination of RT by the stem-loop structures is expected to generate a truncated product of ~920 bases, whereas full-length copying of the template is expected to produce a product of ~1.4 - 1.5 kb in the case of pSP WT1(M1/X) and a product of ~1.9 - 2.0 kb in the case of pSP/flWT1(GNRA).

Please replace the following paragraph starting on page 21, line 2 and ending on line 22

The procedure for generating cDNA libraries has not extensively deviated from the original method of Gubler and Hoffmann (4). A major limitation of the current technology is that a set of products of variable length are often generated during first strand synthesis. Consequently, a number of truncated clones will be present in libraries for any given gene. The difficulty which RT has in transcribing GC-rich regions is well documented. In fact, there are specific RNA structures, called CUUCGG hairpins, which form extraordinarily stable RNA secondary structures capable of blocking RT processivity (2). We have engineered two types of stable stem-loop structures into an Nco I site positioned 918 bp upstream of the Wilm's Tumor WT1 tumor suppressor 3' end (Fig. 2 (SEQ ID NOS:2 and 3)). Plasmid SP/flWT1 contains 433 bp of the 5' untranslated region of WT1 and is ~70% GC rich. Indeed, when cDNA clones for the murine WT1 gene were first isolated, none of the clones were full-length and five of nine clones terminated within 21 nucleotides of each other 182 bases upstream of the ATG codon, suggesting the presence of a strong RT stop signal in this region. The murine WT1 5' end could only be obtained by genomic DNA sequencing (Pelletier et al., 1991, Genes Dev. 5, 1345-1356). We have used in vitro generated WT1 transcripts (ranging in size from ~1.4 - 2.0 kb) to elucidate and optimize conditions which are most effective in allowing RTs of various sources to proceed through these processivity blocks.